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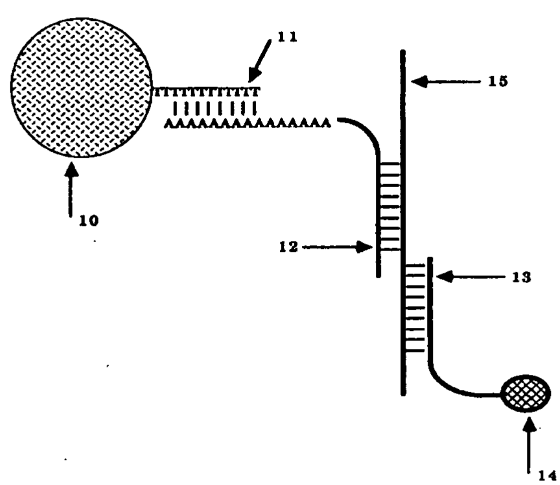
(54) Nucleic acid probes for the detection of mycoplasma fermentans or the aids-associated virus-like infectious agent.

(57) Nucleic acid composition are described for detecting Mycoplasmas associated with AIDS patients. One embodiment of the present invention features a nucleic acid composition capable of hybridizing preferentially to rRNA or rDNA of Mycoplasma fermentans. A further embodiment of the present invention features a nucleic acid composition capable of hybridizing preferentially to rRNA or rDNA of Mycoplasma pirum.

FIGURE 1



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Field of the Invention

This invention relates to the detection of Mycoplasma bacteria which have been described as having an association with the pathogenesis of Acquired Immunodeficiency Syndrome (AIDS). Embodiments of the invention feature nucleic acid compositions and methods for their use for the specific detection and identification of Mycoplasma fermentans and Mycoplasma pirum.

One aspect of the present invention relates to the detection of Mycoplasma fermentans, which is genetically identical to a recently described virus-like infectious agent (VLIA) temporarily designated "Mycoplasma incognitus", which is found with high frequency in patients with AIDS. More specifically, one embodiment of the present invention features nucleic acid compositions and methods for their use for the specific detection or identification of Mycoplasma fermentans and any genetically identical mycoplasma species in clinical and other samples. The nucleic acid compositions feature nucleotide sequences, from 10 to 250 nucleotides, which are capable of hybridizing preferentially to the rRNA and rDNA of Mycoplasma fermentans.

A further aspect of the present invention relates to the detection of Mycoplasma pirum. One embodiment of the present invention features nucleic acid compositions and methods for their use for the specific detection and identification of Mycoplasma pirum and any genetically identical mycoplasma species in clinical and other samples. The nucleic acid compositions feature nucleotide sequences, from 10 to 250 nucleotides, which are capable of hybridizing preferentially to the rRNA and rDNA of Mycoplasma pirum.

Background of the Invention

Mycoplasmas are small wall-less bacteria, primarily isolated from animal sources including humans. There are over 70 members of the genus Mycoplasma, and several related genera which are also characterized by small wall-less bacteria; these are Spiroplasma, Acholeplasma, Ureaplasma, Anaeroplasma, and Asteroleplasma. Only a handful of the species within these genera have been found associated with humans--some presumed to be "normal flora", others occasionally pathogenic, and still others always believed to be clinically significant. Among the mycoplasmas known to be pathogenic, Mycoplasma pneumoniae is historically the most well studied, it is the major infectious agent of primary atypical pneumonia. Nucleic acid compositions and methods relating to Mycoplasma pneumoniae is the subject of an application filed concurrently herewith USSN 673,686, entitled "Nucleic Acid Probes For The Detection Of Mycoplasma Pneumoniae." At least one inventor is common to this application and the present application.

Three other mycoplasma species, which can be isolated from the human genito-urinary tract, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum, are somewhat more enigmatic in the clinical implications of the detection of these organisms in the human body. Ureaplasma urealyticum, for example, although it is implicated in significant and serious human morbidity and mortality, may be found in asymptomatic "normal" individuals as well. The three species, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum may be referred to as the genital mycoplasmas. Nucleic acid compositions and methods relating to genital mycoplasmas is the subject of an application filed concurrently herewith USSN 673,661, entitled "Nucleic Acid Probes For The Detection Of Genital Mycoplasmas." At least one inventor is common to this application and the present application.

In 1989, an additional important pathogenic mycoplasma was reported by S.C. Lo and colleagues at the American Armed Forces Institute of Pathology (Am. Jnl. Trop. Med. Hyg. vol. 40:213:226, 1989, vol 41:364-376, 1989, vol. 41:586-600, 1989). The mycoplasma reported by Lo, temporarily designated "Mycoplasma incognitus" was discovered as a result of a dubious transformation of a cell culture with what was thought to be a viral DNA preparation. Quotation marks are ordinarily used to designate binary bacterial names that are not approved by the governing taxonomic organizations; Mycoplasma incognitus would not be considered an approved name. This mycoplasma, after growth in rich SP-4 media, was the source of various DNA probes and immuno-histochemical test reagents. Testing of samples from AIDS patients, and controls indicated that the mycoplasma VLIA was found in blood and numerous tissues from AIDS patients with high frequency (Lo, et.al. Am Jnl. Trop. Med. Hyg. vol 40, and vol. 41, 1989).

Tests to determine the genetic and serological relatedness of the so-called Virus-Like Infectious Agent, "Mycoplasma incognitus", all point to the designation of Mycoplasma fermentans as the same identical species as the VLIA (Lo, et al. Am. Jnl. Trop. Med. Hyg., vol. 41:586-600, 1989, and Saillard, et al. Res. Virol. vol. 141:385-395, 1990). Polyclonal rabbit antiserum raised against VLIA/"M. incognitus" reacted with only Mycoplasma fermentans and VLIA. In the reciprocal experiments, mule antiserum raised specifically against M. fermentans, reacted only with these same two mycoplasmas (except at very high protein

concentrations). Restriction enzyme patterns, Southern blot analysis, and similar restriction fragment length polymorphism revealed a high degree of identity between "Mycoplasma incognitus" and Mycoplasma fermentans (Lo, et al., Am. Jnl. Trop. Med. Hyg. vol. 4, pg. 586-600, 1989). Saillard et al. report that DNA-DNA homology between VLIA and M. fermentans is approximately 90%. This is fully consistent with Mycoplasma fermentans and "Mycoplasma incognitus" being the same species, and therefore identical at the level of sequence of the ribosomal RNA. By all criteria, genetic and serological, "Mycoplasma incognitus" is a strain of Mycoplasma fermentans, and should be designated as Mycoplasma fermentans VLIA strain. As used herein, Mycoplasma fermentans, Mycoplasma incognitus, VLIA, and Lo's Virus-Like Infectious Agent are synonyms for a single naturally occurring biological entity, correctly known as Mycoplasma fermentans.

In separate reports found in the scientific literature, international AIDS symposia, and the newspapers, L. Montagnier and colleagues at the Institut Pasteur report discovery of mycoplasma bacteria associated with the pathogenesis of AIDS. In one report on the protective activity of antibacterial agents against the cytopathic effects of Human Immunodeficiency Virus (HIV), they speculate that a cofactor, "Mycoplasmas are the likely candidates," is responsible for HIV-induces cell lysis (Lemaitre, et al. Res. Virol. vol. 141:5-16). At the Sixth International Conference on AIDS in 1990, Montagnier reported this mycoplasma to be Mycoplasma pirum (New york Times June 22, 1990 and June 26, 1990).

Additional discussions and assessments of the credibility of the mycoplasma cofactor theory of AIDS have been published in the American Society for Microbiology News (Baseman and Quackenbush, ASM News, 1990), the editor's page of the American Journal of Tropical Medicine and Hygiene (Am. J. Trop. Med. Hyg., vol 42:399-402, 1990), and Science Magazine (K. Wright, vol. 248, 1990). Some additional reports have suggested that the AIDS-associated mycoplasma may be Mycoplasma genitalium. Nucleic acid probes, compositions, and methods for the specific detection of Mycoplasma genitalium may be found in copending USSN 673,661 entitled "Nucleic Acid Probes for the detection of Genital Mycoplasmas," filed concurrently herewith.

The mycoplasmas, such as the ones described above, are fastidious organisms, requiring complex culture media containing peptone, yeast extract, expensive animal sera, and sterol. Growth is relatively slow and reaches low cell densities compared to most bacteria. In addition, atmospheric conditions for cell growth require the addition of carbon dioxide. For these reasons, most clinical laboratories are unable to perform culture isolation of mycoplasmas, and consequently are left with no real ability to diagnose the presence of these important pathogenic bacteria. In fact, there has yet to be a confirmed isolation of M. fermentans VLIA except for the confusing original transformation experiments. There is only one anecodotal report of isolation of Mycoplasma pirum from human, and only a few reports of Mycoplasma genitalium isolation.

Given that mycoplasmas lack cell walls, antibiotics that target the bacterial cell wall, such as penicillin, have no anti-mycoplasma activity. Consequently, it is of importance for a physician to make a diagnosis of the presence of this bacterium, particularly if the clinical presentation is predictive, and prescribe the appropriate antibiotic.

Kohne et al. (Biophysical Journal 8:1104-1118, 1968) discuss a method for preparing probes to rRNA sequences. Kohne et al. do not provide the teaching necessary to make probes to detect Mycoplasma fermentans or Mycoplasma pirum.

Pace and Campbell (Journal of Bacteriology 107:543-547, 1971) discuss the homology of ribosomal ribonucleic acids from diverse bacterial species and a hybridization method for quantitating such homology levels.

Similarly, Sogin, Sogin and Woese (Journal of Molecular Evolution 1:173-184, 1972) discuss the theoretical and practical aspects of using primary structural characterization of different ribosomal RNA molecules for evaluating phylogenetic relationships. Fox, Pechman and Woese (International Journal of Systematic Bacteriology 27:44-57, 1977) discuss the comparative cataloging of 16S ribosomal RNAs as an approach to prokaryotic systematics. Sogin et al., Fox et al. and Pace and Campbell do not provide specific probes useful in assays for detecting the presence and abundance of Mycoplasma fermentans, Mycoplasma pirum or VLIA in samples.

Hogan, et al. (International Patent Application, Publication Number WO 88/03957) describe five probes for the specific detection of Mycoplasma pneumoniae, but fail with respect to the specific detection of Mycoplasma fermentans, Mycoplasma pirum or VLIA.

Woese, Maniloff, Zablen (Proc. Natl. Acad. Sci. USA vol. 77, 1980) examined the partial sequences of selected mycoplasma 16S rRNA, and define the concept of mycoplasma sequence "signature", but fail to teach the art of probe design. They do not discuss M. fermentans.

Rogers, et al. (Proc. Natl. Acad. Sci. USA, vol. 82, 1985) discuss sequences of 5S rRNA, but fail to teach the art of probe design. They do not mention M. fermentans.

Weisburg, et al. (Jnl. Bacteriology, vol. 171, 1989) discuss 16S rRNA sequences of numerous mycoplasmas including Mycoplasma fermentans and Mycoplasma pirum, but do not discuss the VLIA or any aspect of probe design for any mycoplasma.

Ribosomes are of profound importance to all organisms because they serve as the only known means of translating genetic information into cellular proteins, the main structural and catalytic elements of life. A clear manifestation of this importance is the observation that all cells have ribosomes.

Bacterial ribosomes contain three distinct RNA molecules which, at least in Escherichia coli, are referred to as 5S, 16S and 23S rRNAs. In eukaryotic organisms, there are four distinct rRNA species, generally referred to as 5S, 18S, 28S, and 5.8S. These names historically are related to the size of the RNA molecules, as determined by their sedimentation rate. In actuality, however, ribosomal RNA molecules vary substantially in size between organisms. Nonetheless, 5S, 16S, and 23S rRNA are commonly used as generic names for the homologous RNA molecules in any bacterium, including the mycoplasmas, and this convention will be continued herein.

As used herein, probe(s) refer to synthetic or biologically produced nucleic acids (DNA or RNA) which, by design or selection, contain specific nucleotide sequences that allow them to hybridize under hybridization conditions, preferentially to target nucleic acid sequences. The term "preferentially" is used in a relative sense; one hybridization reaction product is more stable than another under identical conditions.

In addition to their hybridization properties, probes also may contain certain constituents that pertain to their proper or optimal functioning under particular assay conditions. For example, probes may be modified to improve their resistance to nuclease degradation (e.g. by end capping), to carry detection ligands (e.g. fluorescein, biotin, and avidin), to facilitate direct or indirect detection (^{32}P, and fluorescent and chemiluminescent agents) or to facilitate their capture onto a solid support (e.g., homopolymer "tails"). Such modifications are elaborations on the basic probe function which is its ability to usefully discriminate between target and non-target organisms in a hybridization assay.

Hybridization is the process by which two partially or completely complementary strands of nucleic acid are allowed to come together in an antiparallel fashion (one oriented 5' to 3', the other 3' to 5') to form a double-stranded nucleic acid with specific and stable hydrogen bonds, following explicit rules pertaining to which nucleic acid bases may pair with one another. The high specificity of probes relies on the low statistical probability of unique sequences occurring at random as dictated by the multiplicative product of their individual probabilities. A minimum of ten nucleotides are necessary in order to statistically obtain specificity and form stable hybridization products. A maximum of 250 nucleotides represents an upper limit of nucleotides in which reaction parameters can be adjusted to determine mismatched sequences and preferential hybridization.

Hybridization conditions are defined by the base composition of the probe/target duplex, as well as by the level and geometry of mispairing between the two nucleic acids. Normal hybridization conditions for nucleic acid of approximately 10 to 250 nucleotides would include a temperature of approximately 60°C in the presence of 1.08 M sodium chloride, 60 mM sodium phosphate and 6 mM ethylenediamine tetraacetic acid (pH 7.4).

Hybridization conditions are easily modified to suit nucleic acid of differing sequences. Factors which may influence the hybridization conditions for a particular nucleic acid composition are base composition of the probe/target duplex, as well as by the level and geometry of mispairing between the two nucleic acids.

Reaction parameters which are commonly adjusted are the concentration and type of ionic species present in the hybridization solution, the types and concentrations of denaturing agents present, and the temperature of hybridization. Generally, as hybridization conditions become more stringent, or less favorable for hybridization, longer probes are required to form stable hybrids.

Summary of the Invention

The present invention features nucleic acid compositions and composition sets and methods for the specific detection or identification of Mycoplasma fermentans and Mycoplasma pirum. As used herein, the term Mycoplasma fermentans and virus like infectious agent (VLIA) are used synonymously.

One embodiment of the present invention features, as a composition of matter, a nucleic acid having approximately 10 to 250 nucleotides, capable of hybridizing to rRNA or rDNA of Mycoplasma fermentans in preference to rRNA or rDNA of non-Mycoplasma fermentans and Mycoplasma pirum bacteria and humans. The nucleic acid composition is useful for detecting Mycoplasma fermentans.

Embodiments of the present invention feature nucleic acids capable of hybridizing to regions of the 16S rRNA or 16S rDNA of Mycoplasma fermentans. The regions of particular interest consist of 50 to 100, 120 to 210, and 420 to 480.

Preferably, the nucleic acid composition is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides within sequences selected from the group of sequences defined probes consisting of 2190, 2193, 2261 and 2273.

One embodiment of the present invention features, as a composition of matter a nucleic acid having approximately 10 to 250 nucleotides, capable of hybridizing to rRNA or rDNA of Mycoplasma pirum in preference to rRNA or rDNA of non-Mycoplasma pirum bacteria and humans. The nucleic acid composition is useful for detecting Mycoplasma pirum.

Embodiments of the present invention feature nucleic acids capable of hybridizing to regions of the 16S rRNA or 16S rDNA of Mycoplasma pirum. The regions of particular interest consist of 100 to 140, 150 to 250, 800 to 860, and 1200 to 1260.

Preferably, the nucleic acid composition is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides within sequences selected from the group of sequences defined by probes consisting of 0001, 0002, 0003, 0004 and 0005.

A further embodiment of the present invention features an article of manufacture for the detection of Mycoplasma fermentans. The article of manufacture comprises a set of nucleic acids comprising at least two nucleic acids. Each nucleic acid is of 10 to 250 nucleotides and of different base sequence composition. Each nucleic acid composition is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 2190, 2193, 2261 and 2273, A set of nucleic acids is particularly suited for detecting Mycoplasma fermentans in a two-probe, sandwich format assay.

A further embodiment of the present invention features an article of manufacture for the detection of Mycoplasma pirum. The article of manufacture comprises a set of nucleic acids comprising at least two nucleic acids. Each nucleic acid is of 10 to 250 nucleotides and of different base sequence composition. Each nucleic acid composition is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 0001, 0002, 0003, 0004 and 0005. A set of nucleic acids is particularly suited for detecting Mycoplasma pirum in two-probe, sandwich format assay.

A further embodiment of the present invention features a methods for detecting the presence of Mycoplasma fermentans. The method comprises the steps of contacting the sample with at least one nucleic acid. The nucleic acid has approximately 10 to 250 nucleotides capable of hybridizing preferentially to Mycoplasma fermentans rRNA and rDNA. The method includes the step of imposing hybridization conditions on the sample which conditions allow the nucleic acid to bind preferentially to Mycoplasma fermentans rRNA and rDNA to form nucleic acid complexes. Finally, the method comprises detecting the nucleic acid complexes as an indication of Mycoplasma fermentans.

Preferably, the nucleic acid of the present method is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 2190, 2193, 2261 and 2273.

A further embodiment of the present invention features a method for detecting the presence of Mycoplasma pirum. The method comprises the steps of contacting the sample with at least one nucleic acid. The nucleic acid has approximately 10 to 250 nucleotides capable of hybridizing preferentially to Mycoplasma pirum rRNA and rDNA. The method includes the step of imposing hybridization conditions on the sample which conditions allow the nucleic acid to bind preferentially to Mycoplasma pirum rRNA and rDNA to form nucleic acid complexes. Finally the method comprises detecting the nucleic acid complexes as an indication of Mycoplasma pirum.

Preferably, the nucleic acid of the present method is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 0001, 0002, 0003, 0004 and 0005.

A further embodiment of the present invention includes a kit for detecting Mycoplasma fermentans. The kit comprises a nucleic acid composition having 10 to 250 base sequences capable of hybridizing to rRNA of rDNA of Mycoplasma fermentans in preference to rRNA and rDNA of non-Mycoplasma fermentans and humans. Typically, kits are comprised of regents, compositions, instructions, disposable hardware and suitable packaging to allow marketing in a convenient assembly.

A further embodiment of the present invention includes a kit for detecting Mycoplasma pirum. The kit comprises a nucleic acid composition having 10 to 250 base sequences capable of hybridizing to rRNA of rDNA of Mycoplasma pirum in preference to rRNA and rDNA of non-Mycoplasma pirum and humans.

Typically, kits are comprised of regents, compositions, instructions, disposable hardware and suitable packaging to allow marketing in a convenient assembly.

The nucleic acid compositions, kits, and methods of the present invention provide the basis for development of valuable nucleic acid hybridization assays for the specific detection of Mycoplasma fermentans and Mycoplasma pirum. The type of samples which may be encountered include sputum, throat swabs, blood, urine, cerebrospinal fluid, skin, biopsy, saliva, synovial fluid, bronchial wash, bronchial lavage, or other tissue or fluid samples from human patients or veterinary subjects. The discovery that probes could be generated with the extraordinary inclusivity and exclusivity characteristics of the present invention with respect to the detection of Mycoplasma fermentans,without necessarily incurring cross-reactivity between other mycoplasma species, and other bacteria was unpredictable and unexpected. It is expected that the probes for Mycoplasma pirum would also share the inclusivity and exclusivity characteristics with respect to the detection of Mycoplasma pirum, as the probes described for Mycoplasma fermentans.

Brief Description of the Tables and Figures

Further understanding of the principles and aspects of the present invention may be made by reference to the Tables and Figures.

Table 1 describes the physical structure of the probes.

Table 2 describes the hybridization behavior of the probes towards the panel of clinically and environmentally representative mycoplasma species.

Figure 1 is a schematic representation of a sandwich assay probe utilizing a capture probe, detector probe and support.

Detailed Description of the Invention

The present invention will be described in detail with respect to nucleic acid compositions having utility as probes for the identification of Mycoplasma fermentans and Mycoplasma pirum. The present discussion will highlight probe development strategy and examples featuring dot blot analysis, sandwich assay formats, in situ applications and amplification techniques for utilizing the nucleic acid compositions of the present invention.

I. Probe Development Strategy

The first step taken in the development of the probes of the present invention involved identification of regions of the 16S rRNA which potentially could serve as target sites for specific nucleic acid probes with the desired sensitivity. This desired sensitivity included finding probe targets unique to Mycoplasma fermentans and Mycoplasma pirum, and thus by analogy the VLIA agents of Lo or Montagnier. For this analysis, precise alignments of mycoplasma 16S rRNA sequences were developed. The essentially complete 16S rRNA sequences of Mycoplasma fermentans and Mycoplasma pirum as well as the 16S rRNA sequences of M. genitalium, M. hominis, M. agalactiae, M. arginini, M. arthritidis, M. bovigenitalium, M. californicum, M. capricolum, M. ellychniae, M. melaleucum, M. gallisepticum, M. hyopneumoniae, M. hyorhinis, M. iowae, M. lipophilum, M. mobile, M. muris, M. mycoides, M. neurolyticum, M. orale, M. pneumoniae, M. pulmonis, M. putrefaciens, M. salivarium, M. sualvi, ten Spiroplasma species' sequences, four Acholeplasma species' sequences, four Anaeroplasma species' sequences, Ureaplasma urealyticum, and one Asteroleplasma sequence. For consideration of other microorganisms capable of deep tissue morbidity, rRNA sequences from several Candida yeasts, Neisseria meningitidis, Staphylococcus aureus, Escherichia coli, and numerous other species were utilized.

Four probe nucleic acid sequences defined by probes 2190, 2193. 2261 and 2273 exhibit exclusivity and inclusivity characteristics toward Mycoplasma fermentans. Probe 2190 is directed to a region of the 16S rRNA extending from 50 to 100. Probe 2193 is directed to a region of the 16S rRNA extending from 130 to 165. Probe 2261 is directed to a region of the 16S rRNA extending from 160 to 200. Probe 2273 is directed to a region of the 16S rRNA extending from 420 to 470. The exclusivity and inclusivity characteristics of the nucleic acid defined by probes 2190, 2193, 2261 and 2273 would be maintained by nucleic acid sharing homology or complementary to at least 90% of a ten nucleotide sequence within the probes.

Five probe nucleic acid sequences defined by probes 0001, 0002, 0003, 0004 and 0005. based on precise alignments of related mycoplasma 16S rRNA sequences, appear as though they will show specificity toward Mycoplasma pirum, analogous to the outstanding exclusivity and inclusivity characteristics

shown by the Mycoplasma fermentans probes. Probe 0001 is directed to a region of the 16S rRNA extending from 100 to 150. Probe 0002 is directed to a region of the 16S rRNA extending from 150 to 200. Probe 0003 is directed to a region of the 16S rRNA extending from 180 to 240. Probe 0004 is directed to a region of the 16S rRNA extending from 800 to 860. Probe 0005 is directed to a region of the 16S rRNA extending from 1200 to 1260. The exclusivity and inclusivity characteristics predictable, by analogy to the probes for Mycoplasma fermentans, of the nucleic acid defined by probes 0001, 0002, 0003, 0004 and 0005 would be maintained by nucleic acid sharing homology or complementary to at least 90% of a ten nucleotide sequence within the probes.

The nucleic acid compositions of the present invention are not to be construed as restricted to the precise specific nucleotides of the named probes. For example, the length of these particular oligonucleotides was optimized for use in the dot blot assay and sandwich assays. Optimal probe length will be a function of the hybridization conditions chosen and each of which can be altered accordingly. In considering sets comprised of more than one nucleic acid, it is desirable that all probes behave in a compatible manner in any particular format in which they are employed. Thus, the exact length of a particular nucleic acid will to a certain extent reflect its specific intended use.

The nucleic acid compositions of the present invention describe sequences which can be employed as oligonucleotide probes, or could be incorporated into larger polynucleotides of either ribonucleic acid or deoxyribonucleic acid. Sequences complementary to the probes described herein can be used as probes to rRNA genes. The preferred probes or their complements can be employed as chain elongation initiators for polymerase chain reaction, sequencing or other applications.

Example 1. Dot-Blot Analysis of Probe Hybridization Behavior

Dot-blot analysis, in accordance with well known procedures, involves immobilizing a nucleic acid or a population of nucleic acids on a filter such as nitrocellulose, nylon, or other derivatized membranes which can readily be obtained commercially, specifically for this purpose. Either DNA or RNA can be easily immobilized on such a filter and subsequently can be probed or tested for hybridization under any of a variety of conditions (i.e., stringencies) with nucleotide sequences or probes of interest. Under stringent conditions, probes whose nucleotide sequences have greater complementarity to the target will exhibit a higher level of hybridization than probes containing less complementarity.

Probes of the present invention were tested in a dot-blot format. One hundred nanograms of RNA, purified by phenol extraction and ethanol precipitation (in some cases, also purified by cesium trifluoroacetate ultracentrifugation) was denatured and spotted on a nylon membrane. Probes were isotopically labelled with the addition of a ^{32}Phosphorous moiety to the 5' end of the oligonucleotide. Hybridization of probes occurred, at a temperature of 60 $^\circ$C in the presence of 1.08 M sodium chloride, 60 mM sodium phosphate, and 6 mM ethylenediamine tetraacetic acid, pH 7.4. Unhybridized probe was removed by washing at a salt concentration one-third of the hybridization condition. The filters were exposed to X-ray film and the intensity of hybridization signals was evaluated after three hours of exposure.

Tables 2 summarizes the behavior of the probes as employed in the present example, and document the specificity of the probes of the present invention.

The representative strain of M. fermentans is the type strain, PG18 (American Type Culture Collection--ATCC 1989). Additional species of bacteria and a few fungi were added to the panel in order to represent the breadth of known bacterial taxa.

In Table 2, "+ + + + " represents strongest hybridization signal after three hours exposure, with slightly lighter signal represented by "+ + + ", diminishing to "+ + ", then "+ ". "+-" is virtually absent, and "-" is indicative of no hybridization of probe to target.

Example 2. Dual Probe Hybridization

The nucleic acid compositions of the present invention have utility in sandwich hybridization assays. Turning now to Figure 1, a sandwich assay is described which features a capture probe 12, a detector probe 13 and a solid support 10. The capture probe 12 ideally would be a bifunctional polynucleotide manufactured by adding a homopolymeric 3' tail to a probe with high target specificity. The tail would, in turn, hybridize to the complimentary homopolymer 11 on a solid surface 10, such as a glass bead or a filter disc. Hybridization of the capture probe 12 to its target 15, in this case Mycoplasma fermentans rRNA or rDNA, would complex the target 15 with the solid support 10. The detector probe 13, advantageously also with some degree of specificity, would be part of a detection scheme relying on radioactivity, fluorescence, chemiluminescence, color. As illustrated, detection probe 13 features a detection moiety 14 which would

report the presence of the entire hybridization complex. The detector probe could potentially be incorporated as an RNA sequence into an amplifiable Q-beta midivariant as described by Kramer and Lizardi (Nature, vol. 339, 1989).

Example 3. Clinical diagnosis of Mycoplasma fermentans or VLIA infection

A clinical sample, blood, a swab, urine, lavage, tissue, or cerebrospinal fluid is processed so as to liberate the total nucleic acid content. The sample, containing disrupted mycoplasmas is incubated in the presence of capture probe, detector probe, and magnetic particle beads which have been derivatized with oligo-Thymidine--as in Example 2--in a chaotropic buffer such as guanidinium isothiocyanate.

If target molecules, Mycoplasma fermentans or VLIA 16S rRNA or rDNA are present in a given sample, a Bead + Capture Probe + Target + Detector Probe hybridization complex is formed, as in Figure 1. The presence of a magnet near the bottom of the reaction tube will cause the magnetic particle + hybridization complex to adhere to the side of the tube enabling removal of the sample matrix, unbound probe, etc. Repeated rehydration and denaturation of the bead + probe + target complex would enable significant background reduction (as described in USSN 922,155, Collins, 1986). In this example, final detection could entail spotting the beads on membrane and assaying by autoradiography. Alternatively, the detector probe could be an amplifiable midivariant probe as described in Example 2.

For this particular assay, the following capture and detector probes are examples of preferred pairs:

Probes 2193 + 2261
Probes 2193 + 2190
Probes 2261 + 2190
Probes 2261 + 2273

Example 4. Clinical diagnosis of Mycoplasma pirum or Montagnier's AIDS-associated mycoplasma infection

A clinical sample, blood, a swab, urine, lavage, tissue, or cerebrospinal fluid is processed so as to liberate the total nucleic acid content. The sample, containing disrupted mycoplasmas is incubated in the presence of capture probe, detector probe, and magnetic particle beads which have been derivatized with oligo-Thymidine--as in Example 2--in a chaotropic buffer such as guanidinium isothiocyanate.

If target molecules, Mycoplasma pirum or AIDS-associated mycoplasma rRNA are present in a given sample, a Bead + Capture Probe + Target + Detector Probe hybridization complex is formed, as in Figure 1. The presence of a magnet near the bottom of the reaction tube will cause the magnetic particle + hybridization complex to adhere to the side of the tube enabling removal of the sample matrix, unbound probe, etc. Repeated rehydration of the complex would enable significant background reduction, as described in Examples 2 and 3.

For this particular assay, the following capture and detector probes are examples of preferred pairs:

Probes 0001 + 0002
Probes 0001 + 0003 Probes 0004 + 0005

Example 5. Clinical diagnosis of Mycoplasma fermentans or VLIA infection from human sample employing polymerase chain reaction amplification of mycoplasma rDNA

Sample processing is designed so as to yield DNA. One of the probes described herein is used in conjunction with the antiparallel complement of one of the probes described herein to enzymatically amplify a segment of Mycoplasma fermentans or VLIA gene encoding mycoplasma rRNA in a polymerase chain reaction. Resultant material can then be assayed in a "sandwich" hybridization (Example 2) with any of the probes described herein. The polymerase chain reaction can, itself, be made either highly specific by employing probe/primers described herein, or the reaction can be made more general using probes such as those described in copending USSN 359,158, and then identifying the amplification product as M. pneumoniae as described in Example 2. Probe 2261 and the reverse complement of Probe 2190 could be used to make a highly specific test for the presence of M. fermentans/VLIA followed by a confirmatory hybridization employing Probe 2193. Similarly, nucleic acid compositions relating to Mycoplasma pirum can be used in a polymerase chain reaction. Resultant material can then be assayed in a "sandwich" assay.

Example 6. In situ hybridization as a cytological stain

The probes of the present invention can be employed as cytological staining reagents specific for Mycoplasma fermentans and Mycoplasma pirum. For example, a blood or tissue specimen is applied to a microscope slide. After appropriate fixation and lysis, hybridization of probes is carried out in situ. In this example, mycoplasmas could be visualized in a specimen by fluorescently labelling Probes 2193 or 2261 and examining the slide using a fluorescent microscope, looking for small fluorescent bodies.

Example 7. Confirmation of presumptive mycoplasma species identification following culture

Following a standard cultivation step for Mycoplasma fermentans, Mycoplasma pirum or VLIA, for example on H-agar plates, SP-6 broth, 10B broth, or A8 agar, a colony or liquid culture is tested for the presence of one of Mycoplasma fermentans, Mycoplasma pirum or VLIA by employing sandwich hybridization assays as described in Examples 2 and 3.

Table 1

Mycoplasma fermentans (Lo's Virus-Like Infectious Agent) 16S rRNA Probes

***Mycoplasma fermentans*/VLIA Probe 2190 (32mer 53% G+C)**

5'-GCC GCT AAG GTA TTG CTA CCT TCG CTC GAC AT-3'

***Mycoplasma fermentans*/VLIA Probe 2193 (33mer 42% G+C)**

5'-TTG AAA TTA TCG ACT GTT TCC AGT CGC TAT GCC-3'

***Mycoplasma fermentans*/VLIA Probe 2261 (34mer 36% G+C)**

5'-TTC CGT AAT CTT CAT GCG AAA ACT ACG AGT ATT T-3'

***Mycoplasma fermentans*/VLIA Probe 2273 (43mer 35% G+C)**

5'-AAT CAT TTC CTA TTC TGT CTT TTC TTC CCT (continues)
TAC CAC AGA AGT T-3'

Table 1 (Continued)

Mycoplasma pirum (Montagnier's Virus-Like Infectious Agent) 16S rRNA Probes

Mycoplasma pirum/VLIA Probe 0001 (32mer 47% G+C)

5'-TTA TCC CCC ACT GTA GGG TAG ATT GGA TAC GT-3'

Mycoplasma pirum/VLIA Probe 0002 (34mer 35% G+C)

5'-TTC TCA TGC GAT AGT ACA TTT GTT ATG CGG TAT T-3'

Mycoplasma pirum/VLIA Probe 0003 (46mer 43% G+C)

5'-CCC TCA TCC TAT AGC GGT CCA AAC GGA CCT (continues)
TTA AAA TGT TTC TCA T-3'

Mycoplasma pirum/VLIA Probe 0004 (38mer 53% G+C)

5'-AGC TAC GAC ACC GAG GCG TTT ACC CCG ACA (continues)
TCT AAC AT-3'

Mycoplasma pirum/VLIA Probe 0005 (40mer 50% G+C)

5'-GCT CCA CCT TGC GGA TTC GCA ACT GTT TGT (continues)
AAT GGC CAT T-3'

Table 2

Mycoplasma fermentans specific probes
Targeting the 16S rRNA

Strain designation	Genus/Species	2190	2193	2261	2273
FH-300	Mycoplasma pneumoniae	-	-	-	-
PI1428	Mycoplasma pneumoniae	-	-	-	-
TW10-5P	Mycoplasma pneumoniae	-	-	-	-
TW10-6P	Mycoplasma pneumoniae	-	-	-	-
TW48-5P	Mycoplasma pneumoniae	-	-	-	-
R32-P	Mycoplasma pneumoniae	-	-	-	-
TW8-6P	Mycoplasma pneumoniae	-	-	-	-
TW25-40	Mycoplasma pneumoniae	-	-	-	-
TW14-4	Mycoplasma pneumoniae	-	-	-	-
TW11-4	Mycoplasma pneumoniae	-	-	-	-
33530 G-37	Mycoplasma genitalium	-	-	-	-
TW10-5G	Mycoplasma genitalium	-	-	-	-
UTMB-10G	Mycoplasma genitalium	-	-	-	-
25960	Mycoplasma pirum	-	-	-	-
19610	Mycoplasma gallisepticum	-	-	-	-
23114 PG21	Mycoplasma hominis PG21	+	-	-	-
15718 KS-1	Mycoplasma putrefaciens	-	-	-	-
PG18	Mycoplasma fermentans	++++	++++	++++	++++
33552	Mycoplasma iowae	-	-	-	+-
KD735	Mycoplasma pulmonis	-	-	-	+-
	Mycoplasma muris	-	-	-	+-
10	Mycoplasma hominis	+	-	-	+-
93	Mycoplasma hominis	+	-	-	+-
132	Mycoplasma hominis	+	-	-	+-
183	Mycoplasma hominis	+	-	-	+-
23206 PG8	Acholeplasma laidlawii	-	-	-	-
10	Ureaplasma urealyticum	-	-	-	-
IG 3224	Citrobacter freundii	-	-	-	-
IG 3157	Escherichia coli	-	-	-	-
33391	Haemophilus influenzae	-	-	-	-
13077	Neisseria meningitidis A	-	-	-	-
15955	Agrobacterium tumefaciens	-	-	-	-
7757	Desulfovibrio desulfuricans	-	-	-	-
33560	Campylobacter jejuni	-	-	-	-
13124	Clostridium perfringens	-	-	-	-
	Spirochaeta aurantia	-	-	-	-
25285	Bacteroides fragilis	-	-	-	-
	Stool RNA	-	-	-	-
	Wheat Germ	-	-	-	-
	Human CaSKi	-	-	-	-
18804	Candida albicans	-	-	-	-
14116	Cryptococcus neoformans	-	-	-	-

Sequence Listing

(1)GENERAL INFORMATION:
(i)APPLICANT: Weisburg, William G.
Pelletier, Dale A.
(ii)TITLE OF INVENTION: NUCLEIC ACID PROBES FOR THE DETECTION OF MYCOPLASMA FERMENTANS OR THE AIDS-ASSOCIATED VIRUS-LIKE INFECTIOUS AGENT
(iii)NUMBER OF SEQUENCES: 9
(iv)CORRESPONDENCE ADDRESS:
(A)ADDRESSEE: GENE-TRAK SYSTEMS
(B)STREET: 31 New York Avenue
(C)CITY: Framingham
(D)STATE: Massachusetts
(E)COUNTRY: USA
(F)ZIP: 01701
(v)COMPUTER READABLE FORM:
(A)MEDIUM TYPE: 3.5 inch/800K bytes-Macintosh formatted diskette
(B)COMPUTER: Macintosh II
(C)OPERATING SYSTEM: Macintosh 6.07
(D)SOFTWARE: MacWrite (MacWrite and Text-only Files)
(vi)CURRENT APPLICATION DATA:
(A)APPLICATION NUMBER: 07/673,687
(B)FILING DATE: MARCH 22, 1991
(C)CLASSIFICATION: (NONE AVAILABLE AS OF YET)
(vii)PRIOR APPLICATION DATA:
(A)APPLICATION NUMBER: N/A
(B)FILING DATE: N/A
(viii)ATTORNEY/AGENT INFORMATION:
(A)NAME: Janiuk, Anthony J.
(B)REGISTRATION NUMBER: 29,809
(C)REFERENCE/DOCKET NUMBER: F-121
(ix)TELECOMMUNICATION INFORMATION:
(A)TELEPHONE: (508) 872-3113
(B)TELEFAX: (508) 879-6462
(C)TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID SEQUENCE

(C) STRANDEDNESS: SINGLE STRANDED

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA PROBE

(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA FERMENTANS 16S RIBOSOMAL RNA.

(ix) FEATURE:

(A) NAME/KEY: GTS OLIGO NUMBER 2190

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCCGCTAAGG TATTGCTACC TTCGCTCGAC AT 32

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID SEQUENCE

(C) STRANDEDNESS: SINGLE STRANDED

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: DNA PROBE

(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA FERMENTANS 16S RIBOSOMAL RNA.

(ix) FEATURE:

(A) NAME/KEY: GTS OLIGO NUMBER 2193

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTGAAATTAT CGACTGTTTC CAGTCGCTAT GCC 33

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID SEQUENCE
(C) STRANDEDNESS: SINGLE STRANDED
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: DNA PROBE
(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA FERMENTANS 16S RIBOSOMAL RNA.
(ix) FEATURE:
(A) NAME/KEY: GTS OLIGO NUMBER 2261

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCCGTAATC TTCATGCGAA AACTACGAGT ATTT 34

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID SEQUENCE
(C) STRANDEDNESS: SINGLE STRANDED
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: DNA PROBE
(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA FERMENTANS 16S RIBOSOMAL RNA.
(ix) FEATURE:
(A) NAME/KEY: GTS OLIGO NUMBER 2273

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATCATTTCC TATTCTGTCT TTTCTTCCCT TACCACAGAA GTT 43

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID SEQUENCE
(C) STRANDEDNESS: SINGLE STRANDED
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: DNA PROBE
(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA PIRUM 16S RIBOSOMAL RNA.
(ix) FEATURE:
(A) NAME/KEY: GTS OLIGO NUMBER 0001

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTATCCCCCA CTGTAGGGTA GATTGGATAC GT 32

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID SEQUENCE
(C) STRANDEDNESS: SINGLE STRANDED
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: DNA PROBE
(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA PIRUM 16S RIBOSOMAL RNA.
(ix) FEATURE:
(A) NAME/KEY: GTS OLIGO NUMBER 0002

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTCTCATGCG ATAGTACATT TGTTATGCGG TATT 34

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID SEQUENCE
(C) STRANDEDNESS: SINGLE STRANDED
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: DNA PROBE
(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA PIRUM 16S RIBOSOMAL RNA.
(ix) FEATURE:
(A) NAME/KEY: GTS OLIGO NUMBER 0003

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCCTCATCCT ATAGCGGTCC AAACGGACCT TTAAAATGTT TCTCAT 46

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID SEQUENCE
(C) STRANDEDNESS: SINGLE STRANDED
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: DNA PROBE
(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA PIRUM 16S RIBOSOMAL RNA.
(ix) FEATURE:
(A) NAME/KEY: GTS OLIGO NUMBER 0004

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGCTACGACA CCGAGGCGTT TACCCCGACA TCTAACAT 38

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID SEQUENCE
(C) STRANDEDNESS: SINGLE STRANDED
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: DNA PROBE
(A) DESCRIPTION: THIS SEQUENCE IS USEFUL AS A DNA PROBE OR AS AN RNA PROBE FOR THE SPECIFIC DETECTION OF MYCOPLASMA PIRUM 16S RIBOSOMAL RNA.
(ix) FEATURE:
(A) NAME/KEY: GTS OLIGO NUMBER 0005

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCTCCACCTT GCGGATTCGC AACTGTTTGT AATGGCCATT 40

Claims

1. A nucleic acid having approximately 10 to 250 nucleotides, capable of hybridizing to rRNA or rDNA of mycoplasmas associated with Acquired Immunodeficiency syndrome, in preference to rRNA or rDNA of non-mycoplasma bacteria and humans.

2. The nucleic acid of claim 1 wherein said mycoplasma is Mycoplasma fermentans.

3. The nucleic acid of claim 1 wherein said nucleic acid is capable of hybridizing to a region of 16S rRNA or 16S rDNA of Mycoplasma fermentans which region is selected from the group of regions consisting of 50 to 100, 130 to 165, 160 to 200, and 420 to 470.

4. The nucleic acid of claim 3, wherein said nucleic acid is complementary to or homologous to at least 90% of a sequence comprising any ten consecutive nucleotides within sequences selected from the group of sequences defined by probes consisting of 2190, 2193, 2261 and 2273.

5. The nucleic acid of claim 3, wherein said nucleic acid is complementary to or homologous to the probes 2190, 2193, 2261 and 2273.

6. The nucleic acid of claim 1 wherein said mycoplasma is Mycoplasma pirum.

7. The nucleic acid of claim 6 wherein said nucleic acid is capable of hybridizing to a region of the 16S rRNA or 16S rDNA of Mycoplasma pirum which region is selected from the group of regions consisting of 100 to 150, 150 to 200, 180 to 240, 800 to 860, and 1200 to 1260.

8. The nucleic acid of claim 7 wherein said nucleic acid is complementary to or homologous to at least 90% of a sequence comprising any ten nucleotides with sequences selected from the group of sequences defined by probes consisting of 0001, 0002, 0003, 0004 and 0005.

9. The nucleic acid of claim 7 wherein said nucleic acid is complementary to or homologous to the probes 0001, 0002, 0003, 0004 and 0005.

10. A set of nucleic acids comprising at least two nucleic acids, each nucleic acid having 10 to 250 base sequences capable of hybridizing preferentially to rRNA and rDNA of Mycoplasma fermentans, and

each nucleic acid of different base sequence composition and complementary to or homologous with at least 90% of a sequence comprising any 10 consecutive nucleotides selected from the group of sequences defined by Probes 2190, 2193, 2261 and 2273.

11. The set of nucleic acids of claim 10 wherein at least two nucleic acid compositions are complementary to or homologous with at least 90% of a sequence comprising any 10 consecutive nucleotides selected from the group of sequences defined by probes, and selected from the group of sets, consisting of,
Probe 2193 and Probe 2261;
Probe 2293 and Probe 2190;
Probe 2261 and Probe 2190;
Probe 2261 and Probe 2273.

12. A set of nucleic acids comprising at least two nucleic acids, each nucleic acid having 10 to 250 base sequences capable of hybridizing preferentially to rRNA and rDNA of Mycoplasma pirum, and each nucleic acid of different base sequence composition and complementary to or homologous with at least 90% of a sequence comprising any 10 consecutive nucleotides selected from the group of sequences defined by Probes 0001, 0002, 0003, 0004 and 0005.

13. The set of nucleic acids of claim 12 wherein at least two nucleic acid compositions are complementary to or homologous with at least 90% of a sequence comprising any 10 consecutive nucleotides selected from the group of sequences defined by probes, and selected from the group of sets, consisting of,
Probe 0001 and Probe 0002;
Probe 0001 and Probe 0003; and
Probe 0004 and Probe 0005.

14. A method for detecting the presence of Mycoplasma fermentans in a sample comprising:
a) contacting said sample with at least one nucleic acid which nucleic acid having approximately 10 to 250 nucleotides capable of hybridizing preferentially to Mycoplasma fermentans rRNA and rDNA;
b) imposing hybridization conditions on the sample which conditions allow said nucleic acid to bind preferentially to Mycoplasma fermentans rRNA and rDNA to form nucleic acid complexes; and,
c) detecting said nucleic acid complexes as an indication of the presence of said Mycoplasma fermentans.

15. The method of claim 14 wherein said nucleic acid of said contacting step is complementary to or homologous with at least 90% of a sequence comprising any 10 consecutive nucleotides selected from the group of sequences defined by Probes 2190. 2293, 2261 and 2273.

16. The method of claim 14 wherein said contacting step comprises at least two nucleic acid compositions, each nucleic acid having 10 to 250 base sequences capable of hybridizing preferentially to mycoplasma rRNA and rDNA, each of a different base composition and are complementary to or homologous with at least 90% of a sequence comprising any 10 consecutive nucleotide selected from the group of sequences defined by probes, and selected from the group of sets consisting of,
Probe 2193 + Probe 2261,
Probe 2193 + Probe 2190,
Probe 2261 + Probe 2290,
Probe 2261 + Probe 2273.

17. A method for detecting the presence of Mycoplasma pirum in a sample comprising:
a) contacting said sample with at least one nucleic acid which nucleic acid having approximately 10 to 250 nucleotides capable of hybridizing preferentially to Mycoplasma pirum rRNA and rDNA;
b) imposing hybridization conditions on the sample which conditions allow said nucleic acid to bind preferentially to Mycoplasma pirum rRNA and rDNA to form nucleic acid complexes; and,
c) detecting said nucleic acid complexes as an indication of the presence of said Mycoplasma pirum.

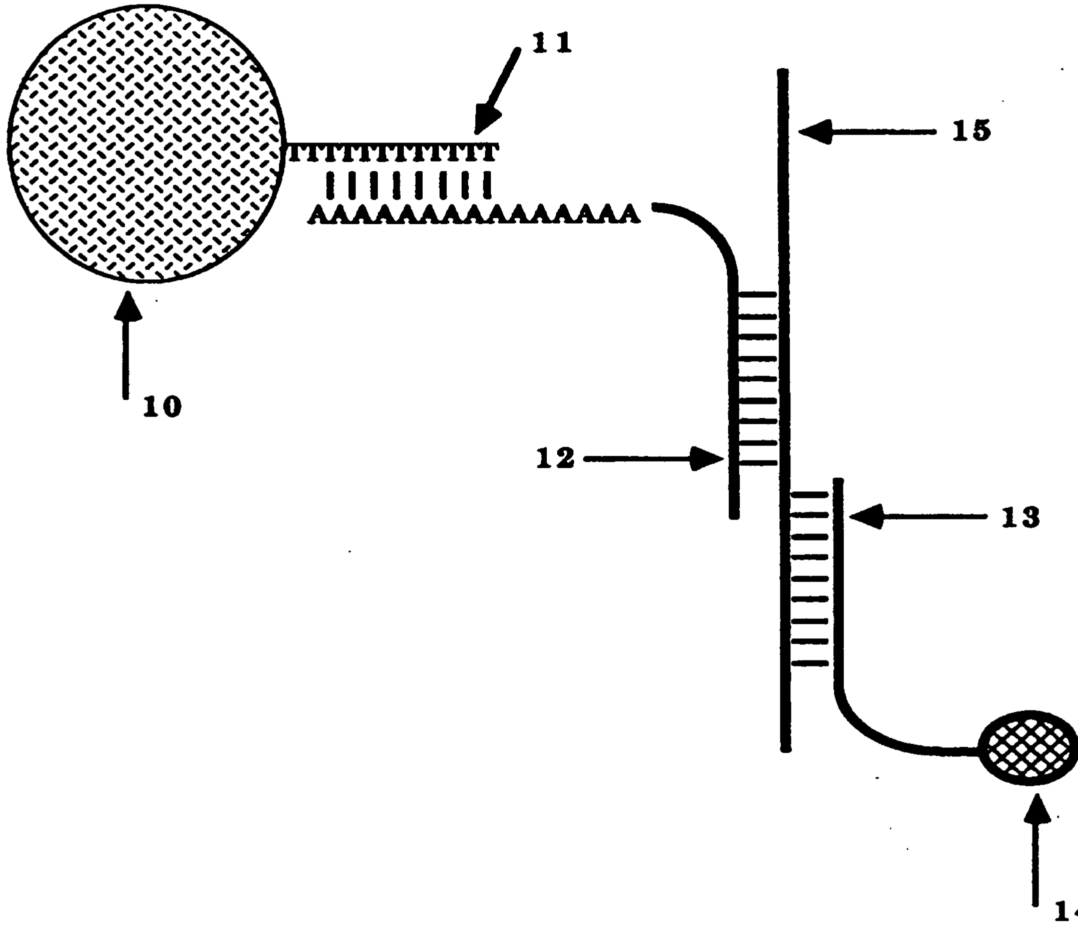
18. The method of claim 17 wherein said nucleic acid of said contacting step is complementary to or homologous with at least 90% of a sequence comprising any 10 consecutive nucleotides selected from the group of sequences defined by Probes 2190, 2293, 2261 and 2273.

19. The method of claim 17 wherein said contacting step comprises at least two nucleic acid compositions, each nucleic acid having 10 to 250 base sequences capable of hybridizing preferentially to mycoplasma rRNA and rDNA, each of a different base composition and are complementary to or homologous with at least 90% of a sequence comprising any 10 consecutive nucleotide selected from the group of sequences defined by probes, and selected from the group of sets consisting of,
Probe 0001 + Probe 0002;
Probe 0002 + Probe 0003; and
Probe 0004 + Probe 0005.

20. A kit for detecting pathogenic Mycoplasma fermentans, comprising a nucleic acid composition having 10 to 250 base sequences capable of hybridizing to rRNA or rDNA of Mycoplasma fermentans in preference to rRNA and rDNA of non-Mycoplasma fermentans and humans.

21. A kit for detecting pathogenic Mycoplasma pirum, comprising a nucleic acid composition having 10 to 250 base sequences capable of hybridizing to rRNA or rDNA of Mycoplasma pirum in preference to rRNA and rDNA of non-Mycoplasma pirum and humans.

FIGURE 1



European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92 30 5124

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	EP-A-0 457 685 (INSTITUT PASTEUR) * page 2, line 1 - line 43 * * page 3, line 54 - page 4, line 26 * * page 5, line 50 - page 6, line 26 * ---	1-3,6,7,14	C12Q1/68 C07K15/00
X	THE JOURNAL OF INFECTIOUS DISEASES vol. 165, no. 3, March 1992, CHICAGO US pages 581 - 585 R.E.HAWKINS ET AL. * the whole document * ---	1,2,14,20	
D,X	RESEARCH IN VIROLOGY vol. 141, 1990, PARIS pages 385 - 395 C.SAILLARD ET AL. * page 388; table I * ---	1,2,17	
X	FEMS MICROBIOLOGY LETTERS vol. 81, 1 June 1991, AMSTERDAM NL pages 37 - 42 A.BLANCHARD ET AL. * page 39 - page 40 * ---	1,6,7,17,21	**TECHNICAL FIELDS SEARCHED (Int. Cl.5)**
D,A	JOURNAL OF BACTERIOLOGY vol. 171, no. 12, December 1989, BALTIMORE US pages 6455 - 6467 W.G.WEISBURG ET AL. ---	1-21	C12Q C07K
D,A	WO-A-8 803 957 (GENPROBE INCORPORATED) * page 1 - page 28 * * page 59 - page 66 * -----	1,14,17	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	07 JUNE 1993	DE KOK A.J.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

European Patent Office

CLAIMS INCURRING FEES

EP 92305124

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

1. Claims 1-5,10,11,14-16,20: Nucleic acids and method for the detection of the AIDS-associated mycoplasma M.fermentans.

2. Claims 6-9,12,13,17-19,21: Nucleic acids and method for the detection of the AIDS related mycoplasma M.pirum

☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

namely claims:

☐ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims: